Effect of prolonged treatment with tyramine on glucose tolerance in streptozotocin-induced diabetic rats

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V. VISENTIN, P. MARQ, S. BOUR, C. SUBRA, D. PRÉVOT, N. MORIN, P. VALET, M. C. MONJE, F. NEPVEU and C. CARPÉNÉ. *Effect of prolonged treatment with tyramine on glucose tolerance in streptozotocin-induced diabetic rats.* J. Physiol. Biochem., **59** (3), 225-232, 2003.

The biogenic amine tyramine has been reported to stimulate *in vitro* glucose transport in adipocytes, cardiomyocytes and skeletal muscle, and to improve *in vivo* glucose utilization in rats. These effects were dependent on amine oxidation, since they were blocked by inhibitors of monoamine oxidase (MAO) and semicarbazide-sensitive amine oxidase (SSAO). We thus tested in this work whether a prolonged treatment with tyramine could improve glucose tolerance in streptozotocin-induced diabetic rats. First, tyramine content of standard rodent chow was determined by HPLC and daily tyramine intake of control rats was estimated to be around 26 µmol/kg body weight. Then, tyramine was administred during 3 weeks in streptozotocin-induced diabetic rats at 29 µmol/kg by daily i.p. injection alone or together with vanadate 0.02 µmol/kg. In another group of diabetic rats, tyramine was subcutaneously delivered at 116 µmol/kg/day by osmotic minipumps. All tyramine treatments resulted in a decrease of the hyperglycemic responses to an i.p. glucose load. Adipocytes isolated from either untreated or treated diabetic rats were sensitive to the stimulation of glucose uptake by tyramine. However, diabetic animals receiving tyramine for three weeks did not recover from their hyperglycemia, hypoinsulinemia and glucosuria. These results show that the improvement of glucose tolerance induced by prolonged tyramine administration occurs in an insulin-depleted model and probably results from peripheral insulin-like actions of the oxidation of MAO/SSAO substrates, such as the stimulation of glucose uptake into adipocytes.

Key words: Semicarbazide-sensitive amine oxidase, Monoamine oxidase, Diabetes, Insulin, Rats.

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Amine oxidation is occuring in many tissues where it has been considered as a way to scavenge exogenous or biogenic amines and to terminate the action of several neurotransmitters (7). Two families of amine oxidases are involved in amine oxidation: the FAD-dependent monoamine oxidases (MAO) and the copper-containing amine oxidase family, essentially represented by semicarbazide-sensitive amine oxidases (SSAO). We have recently reported that adipose and muscular tissues, which are sensitive to insulin, regarding to the stimulation of glucose uptake, express substantial amount of MAO and SSAO (10). In keeping with this, several amines such as tyramine or benzylamine markedly stimulate glucose transport in adipocytes via their oxidation by MAO or SSAO (2, 9). In fact, this stimulation of glucose transport takes place in a manner that partially resembles that of insulin, leading to the translocation of the insulin-sensitive glucose transporter GLUT4 to the cell surface (16). Other insulin-like effects, have been reported for tyramine, which is a substrate for both MAO and SSAO: stimulation of adipose differentiation (5, 8), inhibition of lipolysis (15), and, most importantly, *in vivo* acute improvement of glucose tolerance in conscious rats (10).

The present work aimed at investigating whether prolonged administration of tyramine could improve the impaired glucose disposal of diabetic rats. Therefore, we have studied the capacity of chronically administred tyramine to modify the hyperglycemic response induced by glucose load in rats previously rendered hyperglycemic and insulin-deficient by streptozotocin injection. First of all, we have estimated the amount of alimentary tyramine daily ingested by laboratory rats, by determining the amount of amines in the standard chow with an high-performance liquid chromatographic (HPLC) method. Then, a dose approximatively equivalent to the estimated daily oral intake of tyramine, was daily i.p. injected during 3 weeks to streptozotocin-induced diabetic rats. Other groups of diabetic rats received vanadate at a dose ineffective on its own (0.02 µmol/kg), alone and in combination with tyramine, since vanadate has been reported to improve the insulin-like effect of amine oxidase substrates (16) and the antidiabetic action of chronically administred benzylamine (1). A higher dose of tyramine (116 µmol/kg/day) was also chronically administred during three weeks via mini-osmotic pumps implanted in the dorsal region of streptozotocin diabetic rats.

We report here that the hyperglycemia provocked by i.p. glucose load was reduced in tyramine-treated rats, when compared to untreated diabetic rats, traducing an overall increased glucose utilization. Accordingly, tyramine and other amine oxidase substrates were able, in the presence of vanadate, to stimulate *in vitro* glucose uptake into adipocytes of diabetic rats. Although the prolonged treatments with tyramine did not completely correct the troubles induced by streptozotocin, such as reduced body weight gain, hyperglycemia and hypoinsulinemia, we propose that the improvement of glucose disposal by MAO/SSAO substrates may be useful for the treatment of glucose intolerance.

Materials and Methods

Chemicals.– Tyramine and amines used as standards for HPLC calibration, collagenase, cytochalasin B, fatty-acid-free bovine serum albumin, dansyl chloride, and other reagents were obtained from Sigma-Aldrich (Saint Quentin Fallavier,

France). 2-[1,2-^{3}H]-Deoxyglucose (2-DG, 26 Ci/mmol) was purchased from Perkin Elmer Life science Products (Boston, MA).

HPLC analysis.– After derivatization with dansyl chloride, amines were separated using a gradient elution and a reversed-phase HPLC method with a Waters HPLC system (Milford, MA, USA) coupled to fluorescence detection (λex/em : 350/520 nm) as previously reported (14). A RP-C18 column (5 µm, 200 x 2 mm, 5 µm particle size, 200 Å porosity) (Bishoff Chromatography, Leonberg, D) was used to separate the amines at 25 °C under a linear gradient elution (methanol/water at 0.3 ml/min). Retention times and peak areas were acquired and processed in a Waters Millenium32 workstation. Amine-containing solutions were prepared from rat chow as following: pellets were crushed (2.5 g in 75 ml of water), mixed, and 75 ml of 0.4 M perchloric acid was added; the mixture was stirred for 30 min and centrifuged at 3600 rpm for 5 min. Samples were filtered through a 0.45 µm nylon filter (Millipore) before automated injection.

Animals and tissue sampling.– Male Wistar rats with free access to food and water were rendered hyperglycemic and insulin-deficient by single injection of streptozotocin (65 mg/kg) two weeks before prolonged tyramine treatments consisting in either daily i.p. injection of 29 µmol/kg or implantation in the dorsal region of osmotic minipump delivering 116 µmol/kg/d (Alzet, Palo Alto, CA). After three weeks of treament, animals were subjected to glucose tolerance tests. After sacrifice, the tissues removed were immediately used for adipocyte isolation and subsequent determinations of glucose uptake as previously described (4).

Rats were fasted during 6 hours before the tests. Blood samples were drawen from tail vein of conscious animals at the indicated times before and after glucose load (i.p. bolus of 2 g/kg at time 0). Blood glucose was immediately determined with Glucotrend II glucometer (Roche Diagnostics, Mannheim, D), as already detailed (10).

Statistical analysis.– Results are given as mean ± S.E.M. Statistical significance was assessed by use of Student's *t*-test. NS means no significant difference between the compared samples.

Results

Biogenic amine content of standard chow and daily tyramine intake in rat.– The pellets of the control chow given to rat (Global rodent diet, 2016 R, Harlan, France), containing 16 % protein and 4 % fat were treated as described in Materials and Methods and the content of the five biogenic amines mostly found in food or in animals (14) was determined by HPLC. The amine content (µg/g) was (mean ± SEM of three different determinations): tyramine, 45.3 ± 1.9; spermidine, 40.9 ± 3.2; spermine, 29.6 ± 2.3; putrescine, 14.8 ± 1.7; cadaverine 7.5 ± 0.8. Thus, tyramine was one of the major biogenic amines found in chow pellets while cadaverine was less abundant. Considering that Wistar rats consumed, under our breeding conditions, approx. 20 g of pellets per day, the daily amount of ingested tyramine was estimated to be 26 µmol/kg body weight. We therefore decided to i.p. inject tyramine at 29 µmol/kg/day in diabetic rats during three weeks. A fourfold larger dose (116 µmol/kg/day) was also tested via continuous s.c. administration by osmotic mini-pumps.

Effect of repeated administration of tyramine and vanadate on glucose tolerance in diabetic rats.– Intraperitoneal glucose tolerance tests (IPGTT) were conducted at the end of the three-week prolonged tyramine treatment. The hyperglycemic response provocked by glucose load was markedly reduced in the group of diabetic rats chronically treated with tyramine 29 µmol/kg than in untreated diabetic animals: the peak increase of blood glucose occuring 15-30 min after glucose load was flattened (Fig. 1). The chronic treatment with 0.02 µmol/kg, sodium orthovanadate did not diminish the hyperglycemic response to IPGTT and was unable to improve the antihyperglycemic effect of chronically administred tyramine (Fig. 1). However, the daily administration of tyramine did not correct the reduced weight gain of diabetic animals since, after three weeks of treatment, the body weights were 221 ± 21, and 192 ± 8 g for untreated, and tyramine-treated diabetic rats, vs 296 ± 15 g for normomoglycemic controls (n = 5, p< 0.02). The low dose of vanadate was without any influence on weight gain and the rats treated daily with tyramine and/or vanadate did not exhibited a normalization of their elevated blood glucose (not shown). A higher dose of tyramine was thus tested alone, without combination with vanadate.

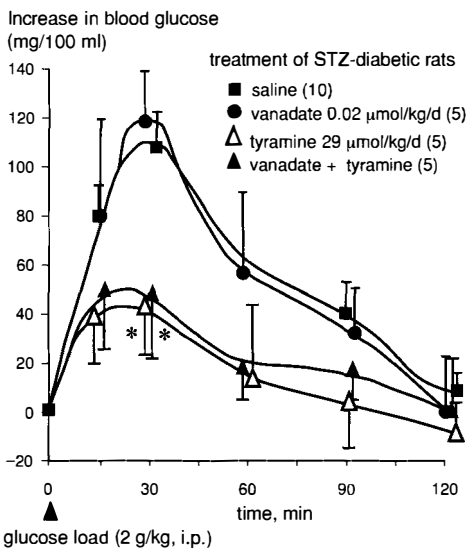

Fig. 1. *Influence of a repeated i.p. treatment with tyramine and/or vanadate on glucose tolerance test in male type 1 diabetic rats.*
After three weeks of treatment, rats were fasted during 6 hours before glucose load (2 g/kg, i.p. bolus). Results are given as increase over blood glucose, which was, at time 0 and in mg/100 ml: 386 ± 13 for untreated diabetic rats (closed squares), 513 ± 33 for vanadate- (closed circles), 400 ± 28 for tyramine- (4 mg/kg, open triangles), and 470 ± 22 for vanadate plus tyramine-group (closed triangles), respectively. Mean ± SEM of the number of rats given in parentheses. Different from untreated diabetic at $^{*}p < 0.05$.

Influence of continuous tyramine administration on glucose tolerance and biological parameters of diabetic rats.– The hyperglycemic response of diabetic rats implanted with osmotic pumps delivering tyramine at 116 µmol/kg/day is shown in Fig. 2. A clear-cut decrease was found in the area under the curve (AUC) of the increase in blood glucose in the rats treated by prolonged tyramine administration, when compared to untreated diabetic rats (arbitrary units were 13.0 ± 0.1 and 4.9 ± 0.1, n = 5, p< 0.01). Accordingly, the rats chronically treated with tyramine exhibited a tendency to disminish their elevated fasting plasma glucose levels at the time of sacrifice (250.4 ± 18.9 vs 328.4 ± 20.2 mg/100 ml, n = 5, p< 0.05) without returning to the levels of normoglycemic controls (65.6 ± 3.4 mg/100 ml). However, chronic treatment with high dosage of tyramine did not correct other defects of streptozotocin-induced diabetes, such as reduced body weight (not shown), severe hypoinsulinemia (0.9 ± 0.4 vs 0.7 ± 0.1 ng/ml, NS), increased plasma

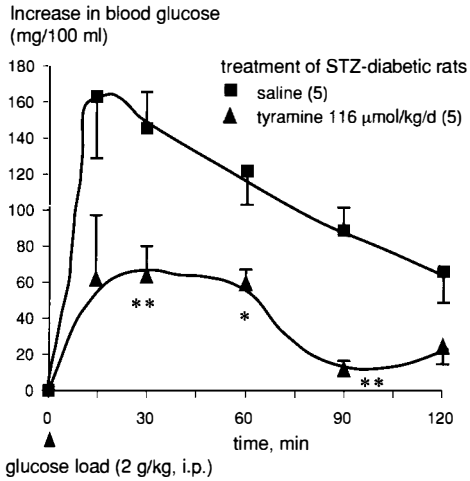

Fig. 2. *Influence of a continuous delivery of tyramine by osmotic pump on glucose tolerance test in diabetic rats.*

Male rats (6-hour fasted) received a glucose load (2 g/kg i.p., arrow). Blood glucose levels are given as increase over blood glucose, which was, at time 0: 412 ± 46 and 415 ± 50 mg/100 ml for untreated diabetic (closed squares) and tyramine-treated rats (116 μmol/kg/d for 3 weeks, closed triangles), respectively. Different from untreated diabetic at $^{*}p < 0.05$; $^{**}p < 0.02$.

lipid peroxidation (5.1 ± 0.4 *vs* 4.7 ± 0.3 μM malonyldialdehyde, NS), and increased urinay glucose excretion (267 ± 14 *vs* 316 ± 32 mg/hour, in treated and untreated diabetic rats, respectively, n = 5, NS). In normoglycemic controls, plasma insulin, plasma malonyldialdehyde, and urinary glucose excretion were: 2.8 ± 0.4 ng/ml, 2.6 ± 0.1 μM, and 0.1 ± 0.1 mg/h, respectively (n = 5, all values different from diabetic at $p<0.001$).

Influence of chronic tyramine treatment on glucose uptake into adipocytes of diabetic rats.– To test whether the antihyperglycemic effect of tyramine observed during IPGTT could be explained by an increased peripheral glucose utilization in severely hypoinsulinic animals, we tested the insulin-like effects of tyramine on glucose transport in adipocytes. Table I shows that tyramine was able to activate hexose uptake in white adipocytes, even in the absence of vanadate. Interestingly, the fold increase over basal induced by 1 mM tyramine or by 100 nM insulin seemed to be greater in treated diabetic rats than either untreated diabetic rats or normoglycemic controls. Vanadate 0.1 mM did not modify basal or insulin-stimulated uptake but potentiated the effect of tyramine in all the experimental groups.

Discussion

Taken together, our observations show that tyramine administration improves

Table I. *Stimulation of glucose uptake (increase over basal uptake) by insulin , tyramine and vanadate in adipocytes from control or diabetic rats.*

		Diabetics	
Group:	normoglycemic	untreated	tyramine-treated
Insulin 100 nM	8.2 ± 1.1	10.2 ± 1.4	14.9 ± 2.9
Tyramine 1 mM	2.1 ± 0.2	2.5 ± 0.4	3.1 ± 0.9
Vanadate 0.1 mM	1.3 ± 0.2	1.0 ± 0.1	1.0 ± 0.3
Vanadate + insulin	8.2 ± 1.0	11.6 ± 1.8	11.9 ± 3.6
Vanadate + tyramine	7.1 ± 1.0	7.3 ± 1.9	9.3 ± 4.0

Basal 2-DG uptake was 1.06 ± 0.17, 1.33 ± 0.27, and 1.35 ± 0.36 nmoles of 2-deoxyglucose/100 mg of lipids/10 min in normoglycemic, untreated and tyramine-treated diabetics, respectively (n = 5, NS). Mean ± S.E.M. of five different determinations.

glucose tolerance in diabetic, insulin-depleted rats. The antihyperglycemic properties of tyramine are likely the consequence of a stimulation of hexose uptake in insulin-sensitive tissues. Even though we did not measure the variations of plasma insulin during IPGTT in the streptozotocin-treated rats, it can be supposed that an indirect effect via stimulation of insulin secretion was not involved in the antihyperglycemic action of tyramine. A stimulation of glucose transport in peripheral tissues by tyramine, as evidenced *in vitro* on fat cells, is therefore expected to occur under *in vivo* conditions. This insulin-like effect could be sufficient to explain the increase in glucose tolerance, although an inhibition of hepatic glucose output or a stimulation of insulin-secretion induced by tyramine could not be excluded since there is very high expression of MAO in liver (10) and since the role of MAO in insulin secretion is still unsolved in the endocrine pancreas (11). It is important to note that the tyramine-induced stimulation of glucose tranport we report here in adipocytes from diabetic rats has been previously oberved also in cardiomyocytes and skeletal muscle from control rats (10). Tyramine could therefore be suspected to lower hyperglycemic response to glucose load by stimulating glucose uptake in all the insulin-sensitive tissues. Moreover, inhibition of lipolysis is another insulin-like action of tyramine found on adipose cells (15) that may facilitate glucose disposal.

The improvement of glucose disposal induced by a chronic treatment with tyramine is in perfect agreement with our previous studies in which an acute administration of tyramine, just before a glucose load, was found to exert antihyperglycemic properties (10). The present data also permit to assess that insulin-like effect of tyramine on glucose diposal persists after repeated administration. This assessment, together with the blockade of tyramine effects by MAO and SSAO inhibitors (10), led to propose that the effect of tyramine is not a receptor-mediated one, which could had been much more prone to desensitization, but is more likely a consequence of its metabolism by detoxifying enzymes.

The role of vanadate remains unclear since it was able to potentiate the acute effect of tyramine on the *in vitro* model, but not the chronic effect of the biogenic amine when tested *in vivo*. Although both doses of vanadate were ineffective in IPGTT or in hexose uptake assays, they were peraphs not equivalent, when compared to the respective tresholds of the diverse insulin mimicking actions of this transition metal. In fact, we have already reported that an i.p. dose of vanadate at 25 μmol/kg is poorly effective in reducing *per se* the hyperglycemic response to IPGTT in rats. The daily dosage used for vanadate treatment in the present study (0.02 μmol/kg) can therefore be considered as being around one thousand fold lower than the treshold for acute *in vivo* antidiabetic effects of vanadate. Regarding stimulation of glucose uptake into rat adipocytes, the dose used to potentiate tyramine effect (0.1 mM) corresponded to the treshold for vanadate in our as well as in other studies (6). Therefore, the observation of a synergism between tyramine and vanadate only in isolated adipocytes, but not *in vivo*, could be explained by the fact that, even perhaps cumulative, only very low doses of vanadate were used for *in vivo* chronic administration.

Instead of increasing vanadate concentration we chosen to increase tyramine dosage. Since it has been previously reported that, at the lower dose tested

(4 mg/kg), an iv. bolus of tyramine induced a transient hypertensive effect that lasted less than 10 min in anaesthetized rats (10), we used a different mode of administration: continous subcutaneous delivery via osmotic minipumps. With this mode of administration, we did not observe any behavioural change in the treated rats. It is important to note that both doses of tyramine tested in this study (4 and 16 mg/kg) were largely lower than the oral dose of 87 mg/kg reported to elicit an increase in blood pressure of 30 mm Hg in rat, which is a reference for clinical risk treshold (3). Moreover, the determination by HPLC of tyramine content in rodent chow allowed to make, at least to our knowledge, the first reported estimation of the daily tyramine intake of a laboratory rat: 26 µmol/kg. This corresponds to approx 1 mg of tyramine ingested/day by a rat of 250 g. Such an amount is found in many foods and beverages: for instance, the amount of tyramine contained in a liter of wine is 0.2-6 mg/l (13). On the basis of our study, one can consider that it is sufficient to increase by two- to eightfold the spontaneous alimentary tyramine intake to obtain a significant effect on glucose disposal. Indeed, the oral ingestion of tyramine led to an important, but not yet well defined, intestinal degradation. Therefore 29 µmoles of i.p. injected tyramine led to an amount of biogenic amine in the peripheral tissues, including adipose depots, much more greater than brought by the dietary amine. In man, endogenous plasma tyramine concentration is around 10 nM under fasting conditions (4). This trace amount makes unlikely that only tyramine participates in a putative interplay between dietary amines and glucose metabolism. However, tyramine and other dietary amines share insulin-mimicking properties and we propose that their effects could be cumulative under *in vivo* conditions rather than competitive until the activity of amine oxidases in adipose tissues or in other peripheral organs does not reach saturation. Thus, it cannot be excluded that it is possible to reach with an oral load a sufficient amount of dietary amines capable to interfere with glucose metabolism as observed here with i.p. or s.c. administred tyramine. To support this view, it can be mentionned that mice fed a diet based on oat variety with a low polyamine content develop smaller lymphosarcoma tumours than animals fed a standard diet (12). This shows that changes in the amount of dietary amines or polyamines can influence the organism maybe via changes in the absorbed amount of amines, escaping from degradation by the intestinal barrier. At the end, the stimulating effects of chonic tyramine challenge on glucose disposal we observed in a model of type I diabetes led us to propose that amine oxidation can constitute a rationale for therapeutic approach of diseases linked to glucose intolerance.

Acknowledgments

This work was partly supported by "Communauté de travail des Pyrénées", by European Union contract QLG1CT1999 00295 "TUNEUP" and by "Pole Aliment-Santé Midi Pyrénées".

V. VISENTIN, P. MARQ, S. BOUR, C. SUBRA, D. PRÉVOT, N. MORIN, P. VALET, M. C. MONJE, F. NEPVEU y C. CARPÉNÉ. *Efecto del tratamiento prolongado con tiramina sobre la tolerancia a la glucosa en ratas diabéticas.* J. Physiol. Biochem., **59** (3), 225-232, 2003.

Se ha descrito que la amina biogénica tiramina estimula *in vitro* el transporte de glucosa en adipocitos, cardiomiocitos y músculo esquelético y mejora la utilización de glucosa

en la rata. Estos efectos son dependientes de la oxidación de la tiramina por la monoamino-oxidasa (MAO) y la amino-oxidasa sensible a semicarbazida (SSAO). En este trabajo, se estudia si un tratamiento crónico con tiramina aumenta la tolerancia a la glucosa en ratas diabéticas por estroptozotocina. El contenido en tiramina del alimento estándar para roedores se determinó por HPLC y se estimó que el consumo diario de tiramina en ratas control era de unos 26 µmol/kg de peso corporal. Por tanto, se administró diariamente durante 3 semanas tiramina por vía i.p. a la dosis de 29 µmol/kg, sola o con vanadato 0.02 µmol/kg a ratas diabéticas por estroptozotocina. Otro grupo recibió tiramina por vía subcutánea mediante minibombas osmóticas liberando 116 µmol/kg/día. Los tratamientos con tiramina han inducido disminución de las respuestas hiperglucemiantes a la sobrecarga de glucosa. En adipocitos aislados de ratas diabéticas, tratadas o no, la tiramina estimula el transporte de glucosa. Sin embargo, los animales diabéticos tratados con tiramina no se recuperaron de su hiperglucemia, hipoinsulinemia y glucosuria. Nuestros datos sugieren que la mejora de la tolerancia a la glucosa inducida por el tratamiento crónico con tiramina se observa en un modelo deficiente en insulina y probablemente se debe a acciones insulinomiméticas. Esto tambien indica que la administración de sustratos de MAO/SSAO podría constituir la base de nuevos tratamientos para mejorar la utilización de la glucosa.

Palabras clave: Amino-oxidasa sensible a semicarbazide, Monoamino-oxidasa, Diabetes, Insulina, Rata.

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